Immunoglobulin A1 Protease Production by *Haemophilus influenzae* and *Streptococcus pneumoniae*

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Bacterial strains of *Haemophilus* species and *Streptococcus pneumoniae* were examined for synthesis of the enzyme immunoglobulin A1 (IgA1) protease. Of 36 *H. influenzae* strains examined, 35 produced IgA1 protease; strains included all six capsular types, unencapsulated variants of types b and d, and untypable *H. influenzae*. Eight *Haemophilus* strains (non-*H.* strains) were studied, and two produced IgA1 protease. All 10 strains of *S. pneumoniae* produced IgA1 protease; these strains included 9 different capsular polysaccharide types and 1 untypable strain. Both IgA1 proteases cleaved myeloma IgA1 and secretary IgA but not myeloma IgA2, IgM, or IgG as determined by immunoelectrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that both enzymes cleaved IgA1 myeloma sera, but not IgA2, into two fragments. The apparent molecular weight of the cleaved fragments was dependent both on the specific IgA1 protease assayed and the specific IgA1 substrate utilized. It is postulated that both carbohydrate variation between the IgA1 substrates studied and the ability of *S. pneumoniae* glycosidases to cleave carbohydrates from glycoprotein offer an explanation for the different fragment sizes observed.

Bacterial immunoglobulin A (IgA) protease was first described by Mehta et al. (14). This enzyme specifically cleaves only human serum IgA1 and secretary IgA, IgA2, IgG, IgM, and IgE are resistant to enzymatic cleavage (14, 17). Cleavage of myeloma IgA1 at a proline-threonine bond in the hinge region results in the formation of Fc and Fab fragments; the Fab fragments of serum IgA1 are unable to bind antigen (18, 20, 21).

A wide variety of microorganisms have been examined for IgA protease production and found to be negative (10). The enzyme is synthesized by many strains of *Streptococcus sanguis* and group H streptococci (10), by virtually all tested strains of *Neisseria gonorrhoeae* (18), and by typable and nontypable *Neisseria meningitidis* strains but not by normal commensal *Neisseria* species (15).

Secretory IgA is the predominant immunoglobulin in colostrum and parotid fluid, and it is felt that IgA may be the predominant immunoglobulin in genital and nasal secretions. Plaut and his co-workers have suggested that IgA protease cleavage of IgA1 in mucosal secretions may be very important in the pathogenesis of infections caused by bacteria which synthesize IgA protease (21). Recently, it has been shown that *N. gonorrhoeae* IgA1 protease can be detected in the vaginal secretions of women with gonorrhea (5).

Both *Haemophilus influenzae* and *Streptococcus pneumoniae* are major pathogens, primarily of children, and it is believed that initiation of systemic infection follows nasopharyngeal colonization. For this reason we elected to examine strains of these organisms for IgA1 protease production.

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MATERIALS AND METHODS

**Bacteria.** A known positive IgA protease strain, *S. sanguis* ATCC 10556, was provided by R. Eisenberg (University of Pennsylvania). A laboratory strain of group A streptococcus was confirmed as not producing IgA protease and was used as a negative control. The pneumococcal strains were provided by Barth Reller. *Haemophilus* strains were provided by the Colorado State Health Laboratories, Barth Reller, Kenneth McIntosh, and Mimi Glode (Children's Hospital, Denver, Co.). In addition to providing numerous *Haemophilus* strains, M. Glode also furnished the Escherichia coli K100 strains.

The clinical origin of most, but not all, of the *H. influenzae* strains was known and included cerebrospinal, blood, and nasopharyngeal specimens. Most of the specimens were from children with invasive *H. influenzae* disease, but some specimens were from "asymptomatic" carriers. Two of the *H. aphrophilus* isolates were presumed to be from patients with endocarditis. The origin of the other *Haemophilus*
strains (non-<i>H. influenzae</i>) is not known. The pneumococcal strains were from patients with systemic pneumococcal disease.

Stock cultures of bacteria were maintained in Todd-Hewitt broth with 30% glycerol at ~70°C. Bacterial strains to be tested for IgA protease were grown on blood agar or chocolate agar in a CO₂ jar at 36°C for 16 to 18 h.

**Immunoglobulins and antisera.** Whole IgA1 and IgA2 myeloma sera were provided by Carlos Abel and Howard Grey (National Jewish Hospital, Denver, Co.), Peter Kohler, Aroop Mangalik, and several private physicians in the Denver area. Purified IgA myeloma and colostal IgA were provided by William Brown and R. Eisenberg. Whole colostrum was received from C. Abel, W. Fuller, and M. Fishut. Concentrated parotid fluid was received from George Revis. The IgA concentrations of the whole myeloma and purified samples ranged from 2 to 15 mg/ml. Routine screening for IgA protease production was done with an IgA1 kappa myeloma serum generously provided by Carlos Abel. This serum had a high IgA1 titer, had no detectable IgG or IgM (by immunoelectrophoresis), and was available in large amounts. The light chains of the IgA1 and IgA2 substrates were typed with antisera to kappa and lambda light chains.

IgM and IgG human immunoglobulins were also assayed for susceptibility to the <i>Haemophilus</i> and <i>S. pneumoniae</i> enzymes. IgM myeloma sera were provided by P. Kohler and R. Eisenberg, and the IgG was Cohn fraction II.

Rabbit antisera to alpha, gamma, mu, kappa, and lambda chains were purchased from Biorad Laboratories.

**IgA protease assay.** Several loopfuls of bacteria to be tested were taken from solid media and evenly suspended in 100 μl of 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 8.1). Usually 10 μl of bacterial suspension was mixed with 10 μl of IgA substrate in a 400-μl microcentrifuge tube with attached cap, and the mixture was incubated in a 37°C water bath for 3 to 4 h; longer incubations were used when secretory IgA was the substrate.

Slides for electrophoresis were prepared with 2% granulated agar (BBL Microbiology Systems). General procedure, buffers, etc. for immunoelectrophoresis were those of Crowle (7). A 3- to 4-μl portion of the incubated bacteria and IgA mixture was placed in a well, and the sample was electrophoresed for 1 h (2 mA/slide, 100 V). Troughs were cut and 50 μl of the appropriate antisera was added. The slides were allowed to develop at 4°C for 2 to 3 days. Slides were processed by the method of Crowle (personal communication) and stained with Coomassie scarlet-brilliant blue R (8).

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), acrylamide slab gels (12.5% gel, 0.3% SDS) with a 5% stacking gel were prepared by the general method of Laemmli (13). After incubation of myeloma proteins with enzyme or buffer, samples were boiled for 3 min in the presence of 2% SDS and 1% mercaptoethanol. Samples were applied to the slab gel and electrophoresed at 10 mA until the dye front reached the bottom of the gel. The gels were stained for 1 h with Coomassie brilliant blue.

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**RESULTS**

**Incidence of IgA protease production.** The incidence of IgA1 protease production among the <i>Haemophilus</i> and pneumococcal strains is shown in Table 1. Protease activity was determined by immunoelectrophoresis. Clearly, IgA1 protease synthesis is a common feature of the <i>H. influenzae</i> strains studied; only one type a strain was negative for protease synthesis. Although the number of other <i>Haemophilus</i> species available for testing was limited, the majority of these strains did not make detectable IgA1 protease. Suspensions of all pneumococci tested, including one unotypable strain, contained IgA1 protease.

Because of the recently recognized association between invasive <i>H. influenzae</i> disease and gut colonization with <i>E. coli</i> K100 strains (11, 27), two <i>E. coli</i> K100 strains isolated from those patients were tested for IgA protease activity; both strains were negative.

**Evidence for IgA1 protease cleavage of IgA1 as determined by immunoelectrophoresis.** The data presented in Fig. 1 indicate that the IgA1 proteases produced by both <i>H. influ</i>
enzae and S. pneumoniae specifically cleaved IgA1 but not IgA2 immunoglobulins. Use of monospecific antisera (Fig. 1B and C) suggested that incomplete cleavage of IgA1 into Fab and Fc fragments had occurred. Anti-kappa antiserum reacts only with the Fab fragment, and anti-alpha chain antiserum reacts only with the Fc fragment, as demonstrated by Mehta et al. (14). Note in slide A that the IgA2 myeloma substrate has IgG and IgM immunoglobulins which are detected with the anti-kappa antiserum. These precipitin lines must be distinguished from the precipitin lines which result from true protease cleavage; the contaminant precipitin lines are qualitatively different from the Fab precipitin lines and are also present in the buffer control. We emphasize this point because if an IgA1 substrate with detectable IgG and IgM is employed, and unequal amounts of substrate are placed in the wells, it is possible to get no contaminant lines in the buffer control and contaminant lines in the incubated mixture.

Haemophilus IgA1 protease seldom cleaved all the IgA1 substrate to completion regardless of the length of incubation. Partial cleavage (Fig. 1C) was more commonly seen, although occasionally complete cleavage into Fab and Fc fragments was observed as evidenced by crossed precipitin lines of nonidentity (14). Pneumococcal IgA1 protease routinely appeared to cleave IgA1 substrates more rapidly than Haemophilus IgA1 protease. Since no attempt was made to quantify the protease assay, it is not known if, with respect to Haemophilus IgA1 protease, pneumococci synthesize more protease or the protease has greater specific activity or there are more pneumococci in the assay tube. The pneumococcal and Haemophilus cleavage products differed with respect to both electrophoretic mobility and diffusion, as a comparison of slides B and C illustrates. We believe that these electrophoretic and diffusion differences may be related to the ability of pneumococcal enzymes to cleave carbohydrate from the heavy chains of IgA1. The rapid diffusion of pneumococcal fragments made it difficult, methodologically, to produce slides for publication which showed separate Fab and Fc fragments which had not diffused into the troughs; use of hyperimmune antisera would have been beneficial.

Both bacterial proteases cleaved secretory IgA (whole colostrum or parotid fluid; purified colostr-
tral IgA1), but not all secretory IgA substrates were cleaved by both enzymes. Cleavage of secretory IgA required a longer incubation period than that with myeloma sera. Comparison of apparent cleavage of colostrum by both IgA1 proteases is shown in Fig. 1D. The pneumococcal IgA1 protease appeared to cleave all secretory IgA substrates most rapidly; protease from *H. influenzae* was the least active against a variety of secretory IgA substrates and *S. sanguis* was somewhat intermediate (data not shown). We have not yet identified the specific cleavage products of secretory IgA.

Identification of secretory IgA cleavage products by immunoelectrophoresis alone is difficult because of the presence of large amounts of IgA1 protease-resistant IgA2 immunoglobulin. Our cleavage patterns are similar to those shown by Genco et al. in slide B of their paper (10). These authors also showed complete cleavage of secretory IgA substrate into Fc and Fab fragments; however, their buffer control showed two precipitin bands referred to as "an experimental artifact." With fresh, structurally intact secretory IgA, which gives a single precipitin band against anti-alpha antiserum, we have never seen cleavage which results in separate Fab and Fc fragments. Similar to the results of Genco et al., we have seen such a pattern with a parotid saliva IgA substrate which was partially degraded and showed two separate precipitin bands when incubated with buffer only.

Neither the *H. influenzae* nor *S. pneumoniae* IgA1 proteases cleaved human serum IgG or IgM (data not shown).

**IgA1 protease cleavage as determined by SDS-PAGE.** As further confirmation that *H. influenzae* and *S. pneumoniae* produced IgA1 protease, we ran polyacrylamide gels to demonstrate the two expected cleavage products from IgA1 myeloma substrates. We were surprised to find the following: (i) the light chains of various myeloma proteins had different mobilities; (ii) assaying a single myeloma substrate, the cleavage products of the *Haemophilus* and pneumococcal IgA1 proteases appeared to be different; (iii) the cleavage products resulting from either *Haemophilus* or pneumococcal IgA1 protease varied among the IgA1 myeloma sera tested; and (iv) the heavy chains of IgA2 proteins had a faster mobility after incubation with pneumococci. Blake and Swanson reported that the cleavage products of *N. gonorrhoeae* IgA1 protease were 30,000 and 33,000 molecular weight as determined by SDS-PAGE (6). Since it is known that the gonococcal IgA1 protease cleaves in the hinge region of the IgA1 immunoglobulin (18), presumably the two cleavage products on SDS-PAGE are the specific Fc and Fd fragments which result from cleavage of the IgA1 alpha chain. Although we have not yet determined the exact site of cleavage for either of our IgA1 proteases, our immunoelectrophoretic and gel electrophoresis data are consistent with cleavage in the hinge region.

The data in Fig. 2 and 3 indicate that the pneumococcal IgA1 protease cleaves IgA1 substrates with the appearance of two bands; one band is very heterogeneous, with an apparent average molecular weight of about 36,000, whereas the faster moving fragment is more homogeneous and has an apparent molecular weight of about 30,000 (this second band is not discernible from the light chains in panel I of Fig. 3). In Fig. 2C one can see that the pneumococcal IgA1 protease has completely cleaved the substrate with the disappearance of the heavy chains; the band above the heavy chains comigrates with the 67,000-molecular-weight

![Fig. 2. SDS-PAGE of IgA1 and IgA2 myeloma immunoglobulins incubated with H. influenzae type b or S. pneumoniae type 1 IgA1 protease.](http://iai.asm.org/)
marker bovine serum albumin and is presumably human serum albumin.

The interaction of pneumococcal IgA1 protease with the IgA2 substrates is particularly interesting. The heavy chains remain intact, but migrate faster than the heavy chains which have been incubated with buffer alone or with Haemophilus IgA1 protease. It is possible that this shift in molecular weight is due to the cleavage of oligosaccharide side chains from the IgA2 proteins by S. pneumoniae glycosidases. In addition to the altered mobility of the heavy chains, two bands can be seen just above the light chain band in Fig. 2F and K; the two bands are not resolved in Fig. 3L, although it is the same substrate as that shown in Fig. 2K. The bands are 32,000 and 33,000 molecular weight with IgA2He and 31,000 and 32,500 molecular weight with IgA2Ke. We suggest that these bands might be the Fc and Fd cleavage fragments which result from the action of pneumococcal IgA1 protease on normal IgA1 present in the IgA2 myeloma sera.

The Haemophilus IgA1 protease, as noted earlier, does not cleave IgA1 substrates as well as the pneumococcal enzyme. In Fig. 2D, one can barely detect the cleaved fragments at molecular weights 33,000 and 36,500. Note that the cleavage fragments obtained with the pneumococcal and the Haemophilus enzyme from the same IgA1 substrate have different apparent molecular weights. When Haemophilus IgA1 protease is incubated with IgA2 immunoglobulin, there is no shift in the molecular weight of the heavy chains. Incubation of Haemophilus IgA1 protease with IgA2 substrates does not lead to cleavage of postulated normal serum IgA1 in the IgA2 myeloma substrates as was seen with the pneumococcal IgA1 protease. In Fig. 3E and F one can more readily see the Haemophilus cleavage products which were so faint in Fig. 2D (same IgA1 substrate). In addition, it can be seen that when Haemophilus IgA1 protease cleaves a different IgA1 myeloma substrate the cleavage products are different. Clearly the fragment in Fig. 3E and F with an apparent molecular weight of about 38,000 is not seen in Fig. 3H, although the 33,000-molecular-weight protein is present. From the increased density of staining below the light chain band in Fig. 3H, it is possible that the second cleavage fragment may be about 29,000 molecular weight. Note that this IgA1 substrate (Tr) is partially degraded and that a band at 33,000 and material moving faster than the light chains can be seen in the buffer control sample (Fig. 3J); spontaneous cleavage of IgA1 myeloma proteins in the hinge region is known to occur (C. Abel, personal communication).

**DISCUSSION**

The results reported here extend the reported incidence of IgA1 protease-binding bacteria to two very important human pathogens, H. influenzae and S. pneumoniae. The incidence and severity of infection with these pathogens in both the pediatric and adult populations are amply documented.

IgA1 protease production appears to be associated with the majority of H. influenzae strains tested. Thus IgA1 protease production cannot, by itself, explain either the increased pathogenicity observed with type b strains (25) or the high rate of nasopharyngeal colonization observed with untypable strains versus the very low rate of colonization seen with encapsulated strains (25). It is not excluded that in vivo interaction between IgA1 proteases from type b and untypable H. influenzae strains with secretory or serum IgA1 or both may be different from the
interaction observed with myeloma sera.

All strains of *S. pneumoniae* studied produced IgA1 protease. Because *S. pneumoniae* strains also have several other enzymes which apparently can alter immunoglobulins by cleaving off all or part of their carbohydrates, we are currently examining the possible effects that carbohydrate cleavage might have on antibody function.

The results obtained with electrophoresis of cleaved myeloma substrates and use of monospecific antisera are consistent with the conclusion that *H. influenzae* and *S. pneumoniae* synthesize IgA1 proteases which cleave only IgA1 substrates into Fab and Fc fragments.

SDS-PAGE was initially performed to confirm the immunoelectrophoretic results. We had not expected to find (i) variations in light chain mobility which influenced the ability to detect cleavage fragments, (ii) alterations in the mobility of IgA2 alpha chains after incubation with the pneumococcus, and (iii) variations in the apparent molecular weights of IgA1 protease-cleaved fragments.

It is known that IgA2 myeloma proteins have complex oligosaccharides attached to asparagine residues in both the Fc and Fd portions of the alpha chain (22). IgA1 myeloma proteins have five N-acetylgalactosamines attached to serine residues in the hinge region; four of the five galactosamines have an attached β-1,3-linked galactose (3). IgA1 myeloma proteins also have complex oligosaccharides attached to asparagine residues; the oligosaccharides are usually found only in the Fc portion of the alpha chain, but can be found in both the Fd and Fc regions, depending on the specific myeloma protein studied (2, 22). It has been shown that IgG myeloma proteins can vary widely with respect to the quantity and quality of carbohydrates found on the light chains and in the Fd region; carbohydrate content of the Fc fragment is quite constant (1). Although IgA myeloma proteins have not been as extensively studied as the IgG myeloma proteins and such carbohydrate variations have not been reported, our SDS-PAGE data are consistent with carbohydrate variations on the alpha chains.

Complex oligosaccharides of the type found on IgA1 molecules can be enzymatically cleaved from glycoproteins by the sequential enzymatic action of neuraminidase, β-galactosidase, exogalactosaminidase, and endogalactosaminidase (12, 23). The pneumococcus synthesizes all four enzymes (12) and might be expected to cleave carbohydrates from the IgA1 substrates studied here, whereas *H. influenzae* is known not to synthesize neuraminidase (16) and therefore could not initiate cleavage of carbohydrates. Since our SDS-PAGE data showed that the alpha chains of IgA2 proteins incubated with pneumococci had a faster mobility than those incubated with buffer or *H. influenzae* alone, we suggest that pneumococcal glycosidases can also cleave complex oligosaccharides from IgA2 proteins. Glycosidase cleavage of carbohydrate from various immunoglobulins is being studied.

In light of the above discussion, our data could be interpreted in the following manner. Pneumococcal IgA1 protease cleaved myeloma IgA1 Sa into a homogeneous fragment of 29,000 molecular weight and a very heterogeneous band with an average apparent molecular weight of 36,500, whereas *H. influenzae* IgA1 protease cleaved the same substrate into relatively homogeneous fragments of 33,000 and 37,000 molecular weight. Although it is recognized that mobilities of glycoproteins in SDS-PAGE gels do not always relate to their carbohydrate content, the faster mobility of the pneumococcus-derived fragments is consistent with cleavage of carbohydrates from these fragments. By analogy with the IgG myeloma data cited above, which showed little carbohydrate variation in the Fc region of heavy chains, we suggest that the faster moving, more homogeneous band might be the Fc fragments. The very heterogeneous, slower moving pneumococcal-derived band may be composed of a family of Fd fragments which contain variable amounts of carbohydrate as a result of cleavage by pneumococcal glycosidases. Alternatively, the reverse situation is perhaps even more plausible. That is, the heterogeneous band could be composed of Fc fragments containing variable amounts of carbohydrate. All IgA1 myeloma proteins studied so far have oligosaccharides in the Fc region (2, 9, 22); carbohydrate in the Fd region is much rarer (22).

Interpretation of the data obtained with myeloma IgA1 Tr is more difficult because the buffer control shows that spontaneous fragmentation of the substrate had occurred, a buffer control containing the same amount of Tr protein as that used for *H. influenzae* was not included, and certain of the protease-cleaved fragments are obscured by the slow moving light chains. Nevertheless, it is clear that the *Haemophilus* and pneumococcus-derived fragments from the same substrate are different, as was seen with IgA1Sα.

Comparison of the pneumococcus-derived fragments from both IgA1 substrates shows differences. The faster migrating fragments from both substrates do not have the same mobility; it is possible that the faster migrating fragment from IgA1Tr may be comigrating with the light
chains. The broad, slow-moving heterogeneous band is a feature common to cleavage of both substrates. Comparison of the Haemophilus-derived fragments from both IgA1 substrates indicates that the 33,000-molecular-weight fragment is common, but the 37,000-molecular-weight fragment from IgA1Sa is not observable with IgA1Tr. We tentatively ascribe these apparent differences in the fragment sizes obtained by the same IgA1 protease (Haemophilus or pneumococcus) acting on different IgA1 substrates to carbohydrate variations of the alpha chains.

We suggested earlier that the two homogeneous fragments observed above the light chains with both IgA2 substrates were the result of pneumococcal IgA1 protease cleavage of normal serum IgA1 in the IgA2 myeloma sera. Patients with IgA myeloma do not suppress synthesis of other immunoglobulins as severely as do patients with IgG myeloma (24). Considering the variations in fragment sizes obtained with IgA1 myeloma sera, it is interesting that the fragments obtained from cleavage of presumed normal serum IgA1 are quite homogeneous and practically identical in both IgA2 sera. It is necessary to exclude the possibility that the fragments detected in the IgA2 myeloma sera are derived from some other component rather than from normal serum IgA1 before speculating on the possible reasons for the observed homogeneity.

Two additional points should be made concerning the data presented in Fig. 3. First, with proteins Sa and Tr, the presumed human serum albumin band is not resolved from the heavy chains. Although several methodological explanations are possible, the validity of data concerning the cleavage products is not an issue. Second, it is quite apparent that the light chains of all three myeloma proteins have different mobilities. Altered mobility of various myeloma light chains has previously been reported (26). It is possible, analogous to some IgG myeloma proteins (1), that some IgA light chains have carbohydrate and some do not. If this were true, we might have expected the pneumococcal glycosidas to alter the mobility of the light chains; no obvious alteration in mobility was seen. However, contamination with IgG and IgM light chains may be obscuring small alterations. Alternatively, it has been reported that lambda-type light chains associated with alpha chains are often unusual with respect to primary structure (22). Proteins Ke and Sa have kappa light chains, and protein Tr has lambda chains. Whatever the explanation(s) for variation in light chain mobility, it is important to choose an IgA1 substrate whose light chains will not comigrate with IgA1 protease-cleaved fragments. Plaut et al. have noted that two-thirds of their myeloma IgA1 substrates had Fab fragments whose mobility in cellulose acetate electrophoresis was similar to the Fc fragment or uncleaved substrate or both (19).

It was mentioned earlier that pneumococcal IgA1 protease appeared to cleave substrates more rapidly than Haemophilus protease. It was also stated that quantitative data are needed to substantiate this qualitative observation. In addition to the variables mentioned earlier, and because we feel the observation is potentially significant, there are at least two other variables that we wish to briefly mention. First, antibodies to Haemophilus protease which inhibit enzymatic activity may be abundant in all IgA1 substrates, whereas antibodies to S. pneumoniae protease are uniformly absent or present at very low levels in all IgA1 substrates. We find this explanation unlikely. Second, pneumococcal glycosidas may expose the IgA1 protease cleavage site by enzymatically cleaving the N-acetylgalactosamine residues in the hinge region, or the complex oligosaccharides found elsewhere on the alpha chain, or both. Although this hypothesis is attractive, it remains highly speculative.

It was mentioned in Results that pneumococcal IgA1 protease appeared to rapidly cleave all secretory IgA substrates examined. Several variables have been mentioned above and in Results to help explain the consistently observed increased cleavage of substrates by pneumococcal protease(s). S. sanguis and H. influenzae IgA1 proteases showed varying degrees of activity against a variety of secretory IgA substrates. This differential activity may indeed be due to different levels of antibody (possibly IgA2) against the cell-bound or free Haemophilus and S. sanguis IgA1 proteases or both in the different secretory IgA specimens.

We are particularly interested in determining the possible in vivo function(s) of S. pneumoniae and H. influenzae IgA1 proteases. Systemic local disease or both with both organisms is thought to be preceded by nasopharyngeal colonization. The complex host and microbial factors involved in the establishment and duration of carriage and the transition from the carrier state to systemic or local invasion or both are not well defined. These events no doubt include initial microbial interactions with secretory IgA, other microbes and host cells. The host’s local and systemic cellular and humoral response to nasopharyngeal or gut colonization or both with haemophil, streptococci, and various cross-reacting microbes may well influence the transition from the carrier state to disease. It is possible that IgA1 protease may modulate coloni-
zation and may be involved in the transition to local or systemic disease or both. We are currently testing this hypothesis.

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LITERATURE CITED